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RELEASE OF POTENTIALLY COLD TOLERANT ALLIGATORWEED FLEA BEETLES--ETC(U)
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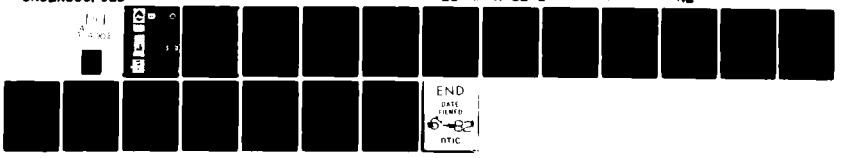

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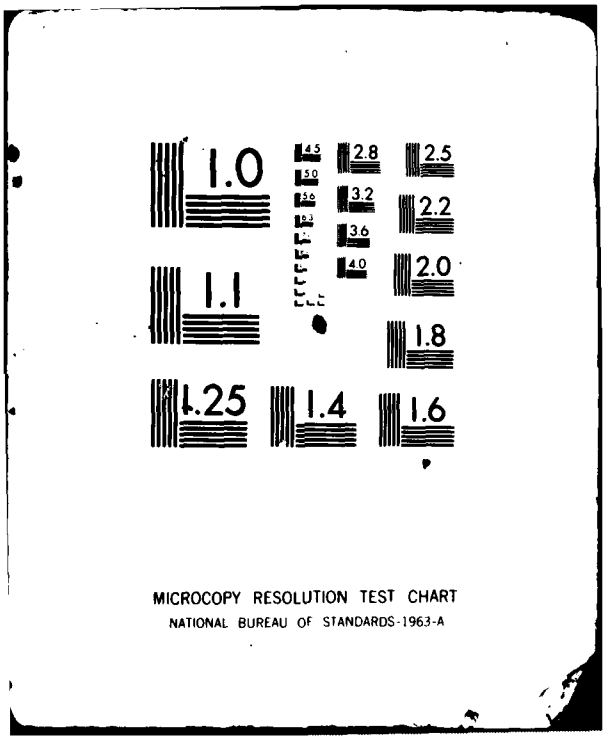
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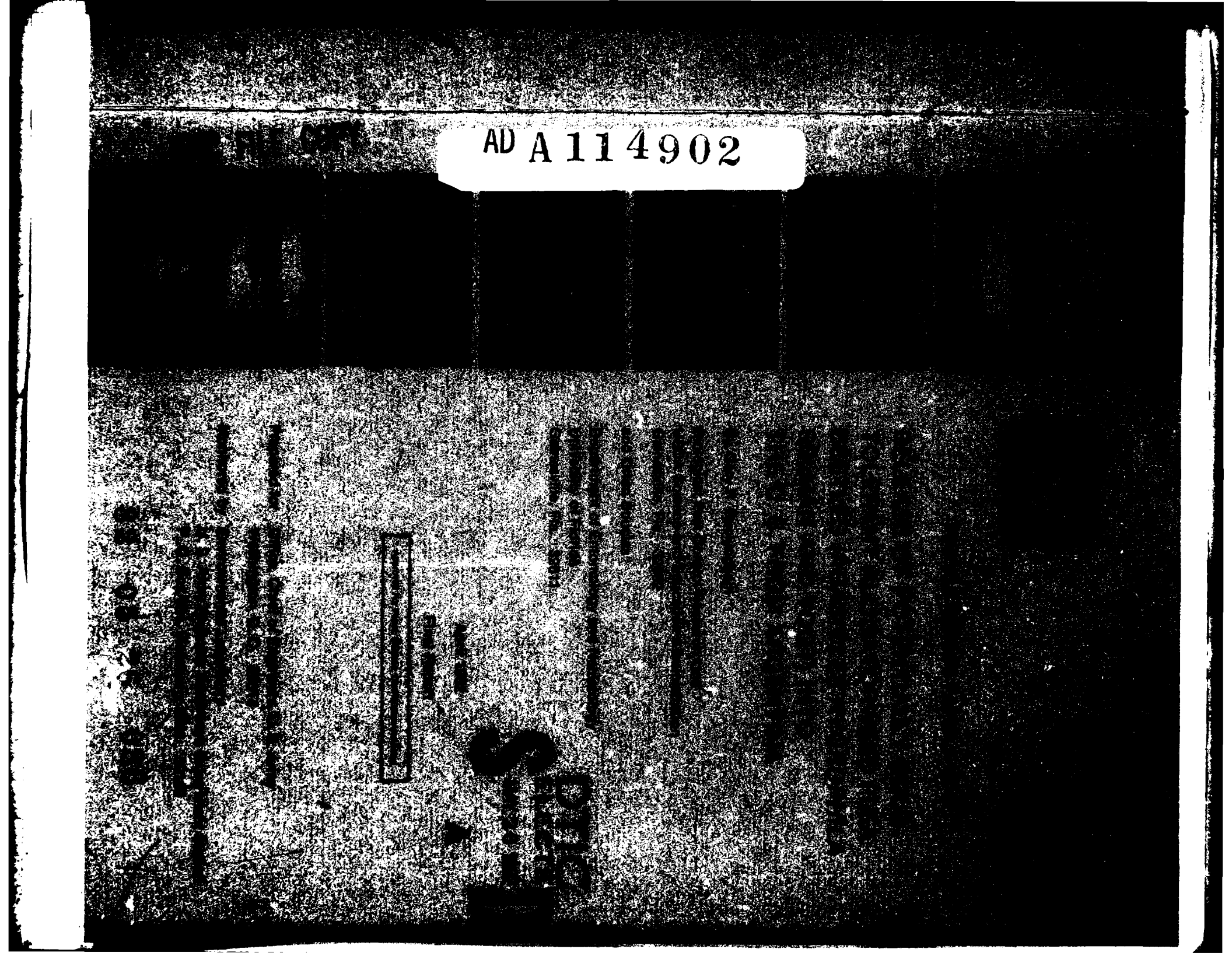

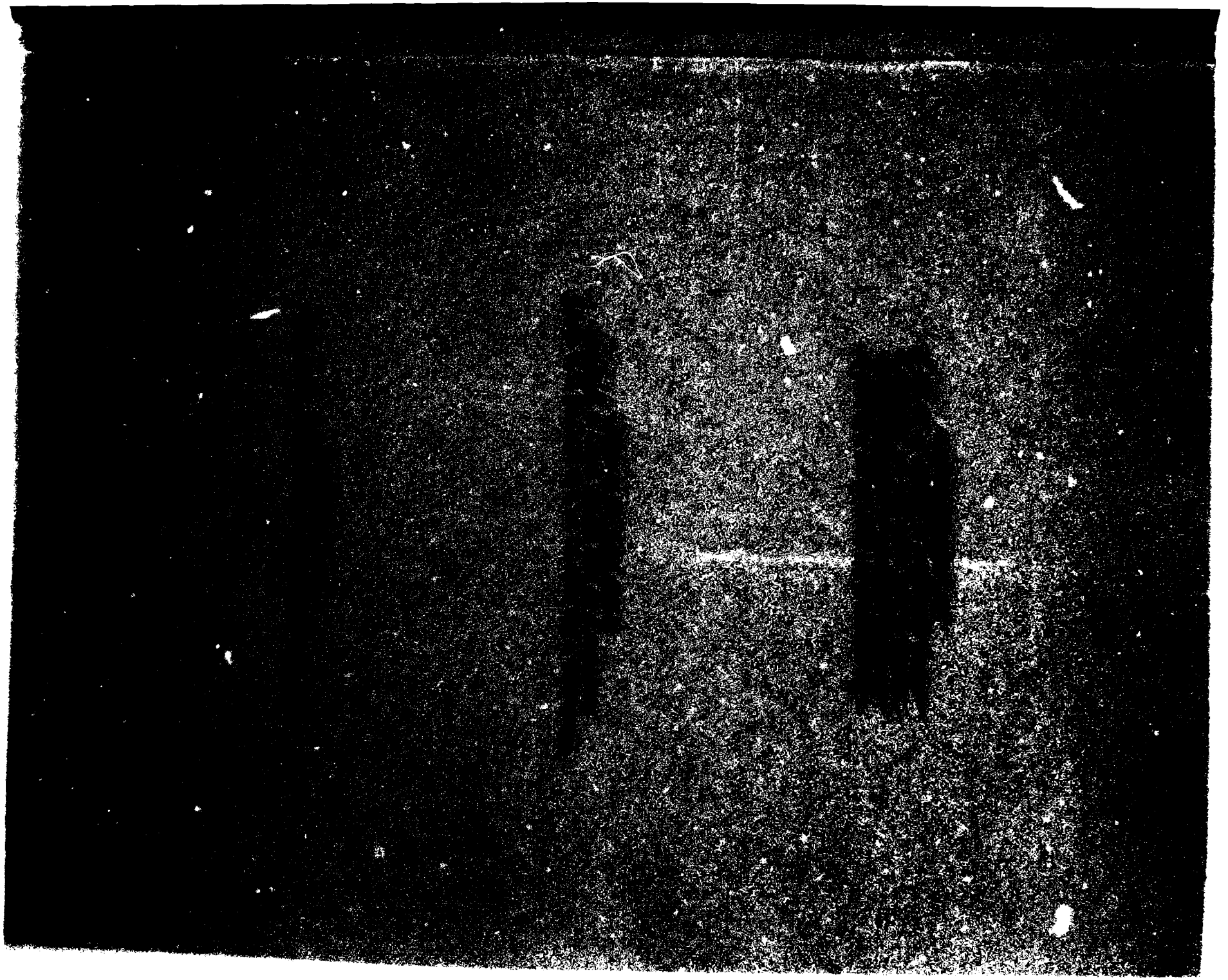

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Alligatorweed flea beetles (Agasicles hygrophila Selman and Vo[illegible]) collected from the southernmost, or coldest, part of their range in A[illegible] and were shipped to the United States. They were reared in quarantine, and specimens from the F_1 generation were examined for entomopathogens, none of which were found. Releases of adults and eggs were made at Gainesville, Fla., and at several locations in North Carolina, South Carolina, and Alabama.

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20. ABSTRACT (Continued)

It was hoped that these beetles would be more cold tolerant than those used in prior releases. Follow-up investigations at the release sites 1 year after release showed overwintering success on only 1 of 10 sites. However, factors other than cold appeared to have eliminated three of the colonies released, so it would be premature to make a determination of whether or not this test population of Agasicles is more cold tolerant than the populations previously released.

Preface

This report presents results from a biological control project being conducted for the Aquatic Plant Control Research Program (APCRP) managed by the Environmental Laboratory (EL) of the U. S. Army Engineer Waterways Experiment Station (WES). The project is being conducted by the U. S. Department of Agriculture (USDA) Science and Education Administration, Biological Pest Control Research Unit, Gainesville, Fla. The purpose of the project is to evaluate the potential of various insects for use in aquatic plant control.

The particular element of the project documented in this report involved evaluation of a possible cold-tolerant strain of the alligatorweed flea beetle, Agasicles hygrophila. Funds to support the investigators involved were provided by the Office, Chief of Engineers, U. S. Army, under appropriation No. 96X3122, Construction General, and by the USDA and the University of Florida.

The principal investigator was Dr. Gary R. Buckingham of the USDA. He was assisted in the conduct of the work and preparation of the report by Dr. Drion Boucias, Insect Pathologist, University of Florida. Mr. Russell F. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), EL, WES, monitored the work and provided valuable assistance in the field portion of the work.

The authors wish to acknowledge Ms. Rosalinda Ferrer, Mr. Jorge Leidy, and Mr. Marga Ferrer of the USDA's Biological Control of Weeds Laboratory in Hurlingham, Argentina, for aiding in the field collection and initial rearing of the insects. Special thanks are due Ms. Bonnie Ross and Ms. Christine Bennett of the Florida Department of Agriculture, Division of Plant Industry (DPI), Biological Control Laboratory, Gainesville, Fla., for rearing the large numbers for release and for helping prepare material for pathogen examination. We also gratefully acknowledge the assistance of all the cooperators who encouraged and aided in the field releases. Dr. Avas Hamon, DPI, and Dr. Badar Munir, University of Florida, reviewed the manuscript and provided helpful suggestions, for which we are grateful.

The work was monitored at WES by Dr. Dana R. Sanders, Sr., WTHG,

and Mr. Theriot under the general supervision of Dr. John Harrison, Chief, EL, and Dr. Conrad J. Kirby, Chief, ERD, and under the direct supervision of Dr. H. K. Smith, WTHG. Manager of the APCRP was Mr. J. L. Decell.

Director of WES during this period was COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

Contents

RELEASE OF POTENTIALLY COLD-TOLERANT ALLIGATORWEED FLEA BEETLES (*AGASICLES HYGROPHILA* SELMAN AND VOGT) INTO THE UNITED STATES FROM ARGENTINA

Introduction

1. The attempt to control alligatorweed [Alternanthera philoxeroides (Mart.) Griseb.] in the Southeastern United States with the imported alligatorweed flea beetle (Agasicles hygrophila Selman and Vogt) from Argentina is often cited as an example of successful biological control of aquatic weeds in the United States.* However, this is true only for Florida and other warm areas in the Southeast. In the colder areas (i.e., South Carolina, North Carolina, and northern Alabama), such attempts have not been successful, apparently because either the beetle does not overwinter successfully or populations that are greatly reduced by high winter mortality are not able to recover fast enough to control the alligatorweed populations.

2. As a possible solution to this problem, Dr. B. David Perkins** suggested that beetles from the southernmost, or coldest, part of their known distribution in Argentina be reintroduced to the United States. This area, near Mar del Plata and Necochea, is about 350 to 425 km (215 to 265 miles) south of Buenos Aires.† An attempt was made in 1974 to bring insects into the United States from an area 259 km (161 miles)

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* Bennett, F. D. 1977. "Insects as Agents for Biological Control of Aquatic Weeds," Aquat. Bot., 3:165-174. Wapshere, A. J. 1979. "Recent Progress in the Biological Control of Weeds," EPPO Bull., 9:95-105.

** Agricultural Research Service (ARS), U. S. Department of Agriculture (USDA), Science and Education Administration, Ft. Lauderdale, Fla., personal communication.

† Vogt, G. B., McGuire, J. U., Jr., and Cushman, A. D. 1979. "Probable Evolution and Morphological Variation in South American Disonychine Flea Beetles (Coleoptera: Chrysomelidae) and their Amaranthaceous Hosts," USDA Technical Bulletin 1593, 148 pp.

south of Buenos Aires, but no insects were released.* All previous U. S. releases had been made with beetles collected at Ezeiza Lagoon near Buenos Aires, except for a small number obtained from Uruguay that were apparently collected at about the same latitude as Buenos Aires.**

3. In February 1979, while surveying in South America for natural enemies of noctuids, the senior author visited the Mar del Plata area to collect alligatorweed flea beetles. The results of that collection, the subsequent events leading to release of the beetles in the United States, and the release site investigations are reported herein.

Procedure

4. Agasicles adults and larvae were collected in Argentina by hand aspiration from the plants or by sweeping with a net. Leaves with eggs were collected by hand. On 27-28 February 1979, all small streams (arroyos) intersecting the highway from Mar del Plata to Necochea and Tres Arroyos, latitude 38-38.5°S, were checked for beetles. High winds and rain hampered collection much of the time. Two adults were found during 30 minutes of sweeping and visual observation at Arroyo Nutria Mansia, Highway 88, about 30 km (18.5 miles) northeast of Necochea. Alligatorweed was present but not abundant in most of the arroyos. Populations of water primrose (Ludwigia sp.) and alligatorweed appear to alternate in abundance during wet and dry years;† during the dry summer of 1979, water primrose was dominant in this area. However, a large, undamaged strand of alligatorweed was found on the banks and in the water at Tres Arroyos.

5. Just north of latitude 38°S and northwest of Mar del Plata, a small colony of beetles was found at the bridge across Arroyo Pantanoso, 20 km (12.5 miles) northeast of Balcarce. In 1-1/2 hours of searching along the bank and while wading, two individuals collected 20 adults,

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* ARS Form 441, File No. FBCL-74-7, Biological Shipment Record - Foreign Source, Collector: H. Cordo.

** Wapshere.

† Vogt, McGuire, and Cushman.

30 to 40 larvae, and four egg masses from the aquatic plants of alligatorweed. Insects were not present on the terrestrial plants along the bank. Many aquatic plants were heavily damaged, while others were not.

6. Arroyo Pantanoso at that location was a small stream about 3 to 4 m (10 to 13 ft) wide and 1.5 m (5 ft) deep. Its low banks overhung the water because the soil had been washed away at the water's surface, and alligatorweed roots were found under this overhang. Most arroyos in the area have low banks since they cut through soil in a relatively flat terrain. These overhanging banks may aid in the winter survival of the beetles by providing a temperate microclimate underneath among the roots. Beetles in northern Florida have been found among alligatorweed roots and accumulated plant debris along the bank of a river on which ice had formed during winter.*

7. Before air shipment to the Biological Control Laboratory, Division of Plant Industry (DPI), Florida Department of Agriculture, in Gainesville, Florida, the field-collected insects were reared and maintained for two generations on bunches of alligatorweed cuttings at the USDA's Biological Control of Weeds Laboratory in Hurlingham, Argentina, in several types of small wooden cages with plastic screening. Use of alligatorweed from a raised, saucer-shaped concrete pool at the Hurlingham laboratory resulted in poor initial egg production by the field-collected adults, but this was quickly rectified by use of plants from a nearby stream. Even though the pool plants were growing in water, the small size of the raised pool and its exposure to the air had produced plants whose upper leaves were similar to those on terrestrial plants. Such leaves are not suitable for oviposition although both larvae and adults were feeding on them. A single airfreight shipment of 20 females, 11 males, and several egg clusters in a cardboard mailing tube inside a cardboard carton was made to the quarantine facility at the Biological Control Laboratory in Gainesville. Alligatorweed cuttings and a damp

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* Charles F. Zeiger, U. S. Army Engineer District, Jacksonville, Fla., personal communication.

organdy-covered sponge provided food and humidity, and excelsior provided footholds.

8. In quarantine, the adults were separated into pairs, and these insects and their progeny were reared in 185-ml (50-dram) plastic vials and 3.8-ℓ (1-gal) glass jars in a room at 24°C (75°F) with fluorescent lighting. A colony of insects initiated from the eggs originally shipped with the adults plus eggs deposited in transit was maintained separately as a reserve. To ensure that the individuals used in the release program were disease-free, F_1 progeny (25 individuals per mating pair) were checked for entomopathogens. Smears of larval, pupal, and adult tissues were prepared by dissection of the organism in distilled water on a microscope slide and subsequently examined at 40 and 100X with a phase-contrast microscope. No definite entomopathogens were detected in the examined tissue samples. The only microorganisms observed were associated with the alimentary tract and were presumed to represent normal gut microflora.

9. Once the beetles (i.e., the adults and progeny of the original shipment) had been determined to be disease-free, most were placed together on alligatorweed in a large cage in a greenhouse. This cage consisted of a basal box, 2.4 by 2.4 by 0.27 m (8 by 8 ft by 10.5 in.) atop 0.9-m (3-ft) legs. The box was waterproofed with liquid fiberglass. Attached to it was a 1-m-high (3.3-ft) wooden frame, covered on the sides with nylon organdy and on the top with a translucent fiberglass panel. Sleeve openings in the center of each side allowed access to the cage. Stems containing pupae were removed periodically to allow space for fresh plant material and placed in other containers and left to mature. Adults from these stems were later returned to the cage. Small populations of the original lines were maintained separately in jars and added occasionally to the cage.

10. On 1 May 1979, the Biological Control Laboratory in Gainesville shipped 37 adults and 86 egg batches by airfreight to Dr. P. Quimby, Jr., USDA Science and Education Administration, Stoneville, Miss., for initiation of a laboratory colony. Meanwhile, alligatorweed stems containing pupae, larvae, and eggs were released in late May at the 34th Street

Bridge over Hogtown Creek in Gainesville and during May and June into laboratory pools at the Biological Control Laboratory. Approximately 4800 to 4900 adults plus 40 egg masses were released in North and South Carolina (Table 1) in June 1979.

11. Prior to the initial field release, beetles were aspirated from the cage and held for 1 to 2 days in small sleeve cages in cool temperature cabinets at about 12°C (54°F) until all of them had been collected. The chilled beetles were then measured volumetrically in a beaker and placed into paper sacks containing alligatorweed cuttings. A small subsample was counted for estimation of beetle numbers. The sacks were carried by car in cold boxes containing chemical ice packs to the field release sites in North and South Carolina. The journey lasted 1 to 1-1/2 days. The actual release was made by opening the sack and placing the alligatorweed cuttings and the sack on an alligatorweed mat.

12. An additional 1300 adults were sent to cooperators (various individuals involved in releasing and maintaining the insects) in these states during July. Prior to the June releases, the general areas had been inspected for beetles or feeding damage. Neither was found, even though beetles had been released in North Carolina at both Lake Waccamaw and at Wilmington during an earlier program.* All of the cooperators indicated that the last few winters had been colder than normal, which may have eliminated the beetle populations.

13. On 26 July 1979, maintenance of the Gainesville laboratory colony was discontinued, and the remainder of the original parental lines, 17 adults and 20 egg batches, which had been maintained separately until combined for shipment, were sent by airfreight to U. S. Army Engineer Waterways Experiment Station (WES) Vicksburg, Mississippi, for initiation of a laboratory colony. A small population remained in the outdoor pools at Gainesville throughout the summer, providing migrants to the surrounding areas. Adults and eggs from the WES colony were

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* Coulson, J. R. 1977. "Biological Control of Alligatorweed, 1959-1972: A Review and Evaluation," USDA, ARS, and U. S. Army Corps of Engineers Technical Bulletin 1593, Washington, D. C., 98 pp.

hand-collected from the rearing cages in a greenhouse on 16 October 1979. The adults and eggs were placed into three waxed paper cups, each of which contained a small amount of wet paper toweling in the bottom to provide moisture. Alligatorweed cuttings provided food and an oviposition substrate. The cups were carried by car to the release sites in Alabama, where the alligatorweed cuttings were removed and placed upon the alligatorweed mats along with the cups (Table 1). The cups were retrieved 1 week later.

14. Release sites were monitored in the fall of 1979 by cooperators to determine if the insects had established a reproducing population prior to the winter.

15. The sites were revisited by the cooperators in the spring and early summer of 1980 to determine if the populations had overwintered. It was decided that if no Agasicles could be found at the release sites at the end of July, the population had not successfully overwintered. It was assumed that any individuals found at the release sites after this date had emigrated from areas further south.

Results and Discussion

16. Overwintering success of Agasicles was determined at only 1 of the 10 sites used in this study (see notes in Table 1).

17. Of the five sites in South Carolina, overwintering was successful only at the Snee Farms site. At the Shaw Air Force Base site, it appeared that the insects did complete one generation in the late summer of 1979, but by the fall of 1979, no individual could be found. No apparent reason for this failure to establish could be determined except that the insects could have emigrated. The remaining three sites had apparently established reproducing populations prior to the winter, but none could be found the following spring or summer. The most likely explanation for this was the cold winter temperatures.

18. There were two release sites in North Carolina. The Lake Waccamaw site was sprayed for mosquito control 2 weeks after liberation of the insects and this apparently destroyed the colony. The release

near Wilmington was unsuccessful, since no Agasicles could be found at the site the following spring or summer. Lack of success was probably due to cold weather since the colony was apparently reproducing in the fall and there was no unusually high water during the winter or spring.

19. There was no overwintering success at any of the three Alabama sites. Two sites, Collier Slough and the effluent canal near the Colbert steam plant, experienced high water (as much as 10 ft above normal) which probably destroyed the colonies. However, the site at Stenson Hollow did not receive unusually high water and its colony also failed to overwinter. This could possibly be due to cold weather.

20. Although the insects overwintered in 1 of the 10 sites, there is insufficient evidence to determine whether or not these insects are more cold tolerant than the populations existing in the more southerly regions of the U. S. These results suggest the need for reintroducing other available alligatorweed biocontrol agents (e.g. Vogtia malloi Pastrana) in these areas, since they appear to be more cold tolerant than Agasicles.*

21. Based on the results obtained from this study and considering that unusually high water and insecticide spraying affected at least three sites, the following recommendations should be considered:

a. Restock the same or other sites with individuals from the site at Snee Farms which was successful and monitor another year.

b. Conduct cold tolerance tests under laboratory conditions of these organisms as compared to existing populations.

c. Reintroduce Vogtia malloi into these more northerly areas and monitor its establishment and population development.

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* Coulson.

Table 1

Releases of *Agasicles hygrophila** in South Carolina, North Carolina, and Alabama in 1979

Date	Locality	No. Released	Release Site	Released by	Notes
			South Carolina		
7 June	Sumter County: Shaw Air Force Base	960-980 A** 20 EM†	Lake No. 1, small golf course lake, approx. 2 ha	R. F. Theriot G. R. Buckingham	Released during light rain with heavy overcast sky. Flat lake margin with large rocks on shore at several areas. Weed is an annual problem. Scattered 2-m fringe of weed along shore. Investigations conducted in the spring and summer of 1980 indicated *Agasicles* did not overwinter
8 June	Charleston County: Snee Farms	960-980 A	Small ponds in housing subdivision	D. Dunseth,†† Santee Public Serv. Comm.	Insects left with W. Melven, USDA, SCS, Columbia, on 7 July 1979. Refrigerated until release. Investigations conducted in the spring and summer of 1980 indicated that *Agasicles* successfully overwintered
13 July	Marlboro County: Bennettsville	350 A	Stream on property of C. C. Griffin	B. W. Anderson,‡ District Conservationist	High streambanks with 10-m mat of weed along margin. Insects shipped from Gainesville by air freight to W. Melven, 11 July 1979. Investigations conducted in the spring and summer of 1980 indicated that *Agasicles* did not overwinter

(Continued)

Table 1 (Continued)

Date	Locality	No. Released	Release Site	Released by	Notes
			South Carolina (Continued)		
13 July	Richland County: Blythwood	300 A	Small pond, approx. 1/2 ha, on property of Victor D. Price	Archie Bickley‡	5-m fringe of weed around pond. Insects shipped with those in Marlboro Co. Investigations conducted in the spring and summer of 1980 indicated Agasicles did not overwinter
13 July	Georgetown County: Peedee River	650 A	Waterfowl impoundment on Weymouth Plantation	M. Prevost,‡‡ Georgetown Co. Health Dept.	Impoundment had wet soil but no standing water. Insects shipped from Gainesville by air freight on 11 July 1979. Investigations conducted in the spring and summer of 1980 indicated Agasicles did not overwinter
			North Carolina		
8 June	Columbus County: Lake Waccamaw	960-980 A	Canal along road on north side of lake in front of J. McNeill cabin	R. F. Theriot G. R. Buckingham	Released late morning on sunny day. Observed flying away during release. Almost solid mat of weed in canal. Banks without overhang. This site was sprayed for mosquite control two weeks after introduction of the colony which destroyed it

(Continued)

Table 1 (Continued)

Date	Locality	No. Released	Release Site	Released by	Notes
			North Carolina (Continued)		
8 June	New Hanover County: Wilmington	1920-1960 A 20 EM	Canals on property of J. Talbert and J. Fox, Oakley Road, north of city on Hwy. 117-133	R. F. Theriot G. R. Buckingham	Released early morning on sunny day. Observed flying away. Large canal system filled with alligatorweed or water primrose-alligatorweed mix. Some overhanging banks. Investigations conducted in the spring and summer of 1980 indicated that Agasicles did not overwinter
			Alabama		
17 Oct.	Colbert County: Wilson Reservoir	60 A 500 EM	Small pond, approx. 2 ha, at Stenson Hollow	R. F. Theriot, T. L. Goldsby, TVA; Eugene Picard, TVA	Pond connected to Reservoir by culvert allowing fluctuations in water depth, entire surface of pond covered with alligatorweed, pond margin ringed with trees. Tree roots and rocks around culvert may provide winter protection. Investigations conducted in the spring and summer of 1980 indicated that Agasicles did not overwinter

(Continued)

Table 1 (Concluded)

Date	Locality	No. Released	Release Site	Released by	Notes
			Alabama (Continued)		
17 Oct.	Colbert County: Pickwick Resevoir	60 A 500 E	Swamp at Collier Slough approx. 2 ha	R. F. Theriot, T. L. Goldsby, TVA; Eugene Picard, TVA	Swamp floods during periods of high water, alligatorweed scattered in small patches, tupelo gum is dominant tree in swamp. Investigations conducted in the spring and summer of 1980 indicated that Agasicles did not overwinter
17 Oct.	Colbert County: Colbert Steamplant Canal	60 A 500 E§	Effluent canal	R. F. Theriot, T. L. Goldsby, TVA; Eugene Picard, TVA	Approx. 3-m fringe of alligatorweed along canal banks, water remains warm throughout winter

* Progeny of insects collected at Arroyo Pantanoso near Balcarce, Argentina, 28 February 1979.
** Adults.
† Egg masses.
†† ARS Form 442, File No. FBCL-79-4. Biological Shipment Record - Quarantine Facility.
‡ ARS Form 442, FBCL-79-7.
‡‡ ARS Form 442, FBCL-79-6.
§ Eggs.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

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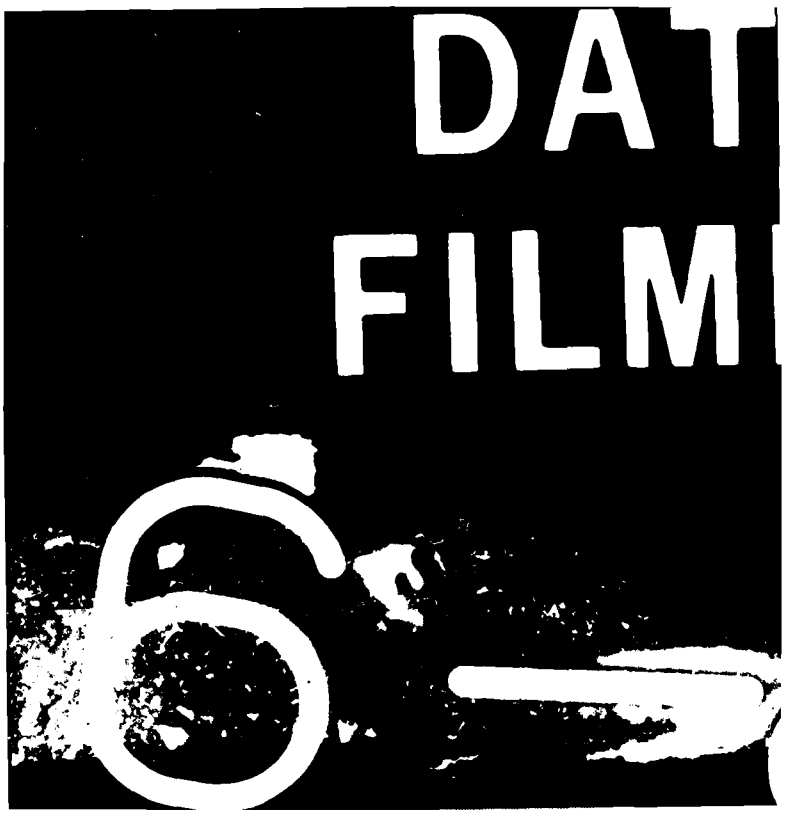
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